spores had been kept, so that it was impossible to determine beyond doubt, whether germination had begun and the embryo had been already partially developed before growth had stopped in the spring. In view of these later observations on fern prothallia, this is by no means improbable, and if this should be the case, heterospory in *Selaginella* would be advanced one step further in the direction of seed-formation.

It is certain that further examination of the archegoniates of our arid and semi-arid regions will reveal other adaptations quite as interesting as those already recorded.

Stanford University,
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THE POLLEN TUBE IN THE CUCURBITACEAE AND RUBIACEAE

By Francis E. Lloyd

In a recently published paper * Longo has given us the very interesting results of his later investigations on the behavior of the pollen tube in the Cucurbitaceae. The close similarity of the facts presented by Longo to those which have been observed by myself in Rubiaceae,† and the parallelism of our conclusions, will, perhaps, warrant a brief comparative statement of our results.

According to Dr. Longo, the ovary in *Cucurbita Pepo* L. is provided with a special conductive tissue which arises, by tangential cell-division, "from the epidermis of the placental ridges and extends uninterruptedly from the style, through the three central laminae, to the ovule." The placentae, of course, fuse along their surfaces with one another, so that the layer of conductive tissue between any two of them is derived from two epi-

* Longo, B. Ricerche sulle Cucurbitaceae e il significato del percorso intercellulare (endotropico) del tubetto pollinico. R. Accad. Lincei, Va, 6 : 523–547. *pl. 1–6.* 1903. ["Presented in December, 1902." Bot. Centralb. 95 : 114. 1904.]

† Lloyd, F. E. The Comparative Embryology of the Rubiaceae. Memoirs Torrey Botanical Club, 8 : 27–112. *pl. 5–15.* 15 F 1902.

dermal cell-layers, and is continuous transversely, as well as longitudinally.

The tissue consists, moreover, of small sub-isodiametric cells, rich in contents, and *not lengthened in any direction* in particular. They lack, therefore, the anatomical characters found generally in stylar conductive tissues, and are in form, at least, like the cells which lie in portions of the path of the pollen tube in *Casuarina*, certain Amentiferae, *Alchemilla* and in a number of other plants.*

The conductive tissue is further extended between the ovules, being interrupted by their funicles, and comes *to abut upon their nucelli*. This is brought about by the circumstance that in *Cucurbita Pepo* the nucellus has the shape of a flask, the long neck of which extends through and protrudes beyond the micropyle, its end coming into contact with the conductive tissue. It thus comes about that there is, in this species, *no free locular space*, but rather a continuous stretch of conductive tissue from the stigma to the embryo sac. What would be inferred, namely, that the pollen tube must therefore have a completely intercellular course, is indeed the case, as Longo has demonstrated.

In the other genera studied, on the other hand, *e. g.*, *Citrullus vulgaris*, Schrad. (which Longo figures), the nucellus has but a short neck, which leaves open a micropylar canal of considerable extent. From these, too, is absent the conductive tissue described for *Cucurbita Pepo*, there being instead an ovarian space. Through this, and through the micropyle, the pollen tube grows freely in its path to the nucellus, where, however, as in many other plants, it must enter upon a short intercellular course in order to reach the embryo sac. It thus appears that, *in two closely related genera in the same family, and indeed in different species of the same genus, the pollen tube differs in its behavior, in that, in the one case its course is throughout intercellular, while in the other it moves freely in a cavity.*

In every other species of this genus, *Cucurbita*, the nucellus is too short to reach quite up to the conductive tissue (*e. g.*, *C. maxima*, *ficifolia*, *foetidissima*) and in these, the pollen tube

* For citations of the pertinent literature, see either of the papers above indicated.

moves freely in the cavity thus formed in reaching the nucellus.

There are, in addition to the above, some further details of interest in regard to the pollen tube. Its path, as described by a number of observers for other plants, lies between the cells of the conductive tissue in all cases. In *Cucurbita*, upon reaching the base of the neck of the nucellus, it enlarges to form a "bulla" of considerable size. Furthermore, this bulla in some cases produces lateral cul-de-sacs, which extend into the surrounding tissues, after the manner of the lateral suspensor tubes in certain orchids * and in the Galieae among the Rubiaceae.† It further appears that the production of these lateral outgrowths of the "bulla" is correlated with the supply of starch in these surrounding tissues, since, when starch is absent, none are formed, and when abundantly present, the tubes are most extensively developed. Their use, therefore, according to Longo, is to reach out after food materials, which he believes are then passed on to the embryo, as a nutritive supply for its growth.

The conclusion that there is a correlation between the growth of the bulla and the presence of starch, the extent of the former being in direct proportion to the presence of the latter, appears to be supported by Longo's observation that, when pollen grains are grown upon gelatine, bullae are formed, while, when they are allowed only moist air, they fail. These results are analogous to those of van Tieghem obtained for *Ricinus* and *Symphytum*. Van Tieghem, however, used gum arabic instead of gelatine. In *Elodea Canadensis*, however, according to R. B. Wylie, ‡ the pollen tubes which enter the ovarian cavity sometimes fail to reach the micropyle, and these produce "cystoids" which are usually found to be quite free in the locule. Wylie further notes that these "cystoids," which, it would seem, so far as we can at present see, are not different from the structures observed by Longo and van Tieghem, are never produced in the tissues,

* Treub, M. Notes sur l'embryogénie de quelques Orchidées. Naturk. Verh. K. Akad. 19 : —. 1879.

† Lloyd, F. E. *Op. cit.*

‡ The Morphology of *Elodea Canadensis*. Bot. Gaz. 31 : 1–22. Ja 1904.

where, without doubt, food materials occur in greater abundance, however little their actual amount, than in the ovarian cavity. We must therefore conclude, in the absence of further evidence that this behavior may not wholly be determined in the manner asserted by Longo.

In discussing the facts above outlined, Longo draws the following conclusions:

1. The behavior of the pollen tube in following an intercellular path is not connected with its inability to grow freely in an open space. This is to be inferred from the fact that in closely related species the behavior in this respect is different according to the continuity of the conductive tissues. Longo has completely demonstrated the truth of his contention, by showing that the pollen of *Humulus Lupulus* L., and of *Cannabis sativa* L.,* in all of which the pollen tube has an endotropic course, germinates in humid air, and pollen tubes are produced. Experimental evidence was obtained in the case of the pollen of certain gymnosperms (*Picea excelsa*, three spp. of *Pinus*) which also germinated and produced tubes in moist air, from which he correctly inferred that the endotropic behavior in angiosperms cannot be due to an inheritance of this trait in the gymnosperms, as was pointed out by Murbeck.† Clearly, we must, as Longo maintains, abandon the position that the phenomena of ectotropism and endotropism are quite distinct from each other.

2. Chalazogamy is not primitive in the angiosperms, and this, or in general, the intercellular behavior, is a later phenomenon, and in this, Longo differs from Treub and Nawaschin, but agrees with Murbeck, who regards such behavior as a physiological peculiarity, and of no meaning from a phylogenetic point of view.‡

3. In plants in which the pollen tube is endotropic, the direction of its growth is determined by the presence of a special substance to which it responds chemotactically. This, Longo adds, is developed within the tissues when the course is endotropic

* Longo, R. *Op. cit.*, p. 21.

† Murbeck, Sv. Ueber das Verhalten des Pollenschlauches bei Alchemilla arvensis. Lands. Univ. Arsskrift, 36: 9. 1901.

‡ Murbeck, Sv. *Op. cit.*

and on their surfaces when the course is superficial. This conclusion is drawn from the circumstances that the pollen tubes of *Humulus* and *Cannabis*, when growing upon a cover-glass in humid air, are indeterminate in the direction of their growth.

We may now turn to the facts concerning the behavior of the pollen tube in the Rubiaceae. The plants which offer these are *Richardsonia pilosa*, *Diodia teres*, and *D. Virginiana*. The ovary in *Richardsonia* is trilocular, in *Diodia*, bilocular. In all three there is a single ovule in each locule, and this is inserted at the top of the basal element of the partition which separates the locules. This partition reaches to the middle height of the ovary, and the separation of the locules from this point on is completed by the stylar elements. The latter has a conductive tissue of elongated cells, which extends to its point of fusion with the basal element of the partition. Up to this point in all three the course of the pollen tube is direct and endotropic. After this, however, it is quite different, in *Richardsonia* and *Diodia teres* on the one hand, and *D. Virginiana* on the other. The ovule in *Diodia Virginiana* has a tract of epidermal conductive cells which are cubical in form, on the outer surface of which is secreted a mucilaginous substance. On the surface of these cells, the pollen tube moves freely till it reaches the micropyle, which it enters and traverses till it reaches the embryo sac. The conductive tissue is confined to a strophiolar outgrowth of the ovule.

In *Richardsonia* and *D. teres*, however, the ovules are provided with a conductive tissue of deeply columnar epidermal cells, with dense protoplasmic contents, and thick but soft and yielding walls. There is, however, no mucilaginous secretion to be found upon their exposed ends. In these plants, the course of the pollen tube is always (*Diodia teres*) or nearly always (*Richardsonia*) completely endotropic. The pollen tube grows between the epidermal cells, as in the Cucurbitaceae, and follows a direct path toward the micropyle at right angles to the direction of least resistance. Aside from the latter fact, we have, it is seen, *two closely related genera, and two species of one of these, in which the behavior of the pollen tube is different, in that, in one species the course is ectotropic, and in the two others, distributed in different genera, the course is endotropic.*

The following conclusions have been inferred by me from the above observations:

1. The form of the cells which compose a conductive tissue can have no importance in directing the pollen tube, though they may deflect it here and there in its course. This is clear from the circumstance that we have in two of the species studied, a conductive tissue, the cells of which are elongated at *right angles to the path* of the tube. We must therefore conclude that the guidance of the pollen tube through the tissue is due to a chemotactic stimulus, thus confirming the view of Molisch and with which Longo finds agreement. In order, however, to determine *direction*, the stimulant must, I have maintained, be distributed differentially, from a center toward which, therefore, the pollen tube must grow. I now believe that the egg cell is the origin of this substance.

2. The behavior of the pollen tube, whether ectotropic or endotropic, is a purely physiological character. When the mechanical conditions make it necessary, as, *e. g.*, in *Cucurbita Pepo*, and as I have shown in *Diodia teres*, the path of the pollen tube is wholly endotropic; when, however, there is a free space to move in, this may be used by the pollen tube, or if the distribution of the chemotactic stimulant is otherwise, the free space may be avoided. By inference, the phylogenetic interpretation of endotropism advanced by Treub and Nawaschin is of no further importance.

For other facts and conclusions, less relevant to our present purpose, the reader may be referred to the original papers. It is of no small interest that almost precisely parallel facts have been brought to light independently by two different observers, and that, equally independently, the same significance has been attached to these facts.

SHORTER NOTES

Evening Primroses. — The evening primroses (*Onagra* or *Oenothera*) are the center of an unusual interest at the present time because of the new species which have been seen to arise